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ORIGINAL ARTICLE

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Prevalence of Enterotoxigenic *S. aureus* in Table Eggs in El-Fayoum City, Egypt

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Abstract
This study was designed to determine the prevalence of enterotoxigenic *S. aureus* in table eggs in El-Fayoum city, Egypt. A total of 250 table egg samples (75 Baladi hens', 75 white farm hens', 75 brown farm hens' and 25 duck egg samples) were collected randomly from poultry farms, groceries, supermarkets, and street vendors in El-Fayoum city, Egypt. Each Baladi hen's egg sample was represented by five eggs, while each farm hen's and duck egg sample was represented by three eggs. The shells and contents of eggs were examined for the presence of *Staphylococcus spp*., coagulase (coa), and staphylococcal enterotoxins (Ses) genes. The obtained results revealed that the examined samples of shells and contents of Baladi hens', poultry farms' (white and brown), and ducks' eggs were contaminated with *Staphylococcus spp.* with incidences of 24.0, 9.3, 5.3, 44.0, 8.0, 2.7, 1.3 and 12.0 %, respectively and coagulase-positive *S. aureus* with the incidences of 16.7, 14.3, 0.0, 18.2, 0.0, 0.0, 0.0 and 33.3 %, respectively. Enterotoxin profiling by PCR proved that two classical enterotoxin genes (Seb and Sed) were produced from three (42.86%) coagulase-positive *S. aureus* strains, as two Baladi hens" eggshells produced Seb and one of the ducks' egg contents produced Sed. The public health hazards of the isolated strains and enterotoxins had been discussed.

Keywords
Enterotoxins, PCR, *S. aureus*, Table Eggs

1. Introduction
Table eggs are devoured worldwide and play an important part of human diet for many reasons, which are the high quality of egg proteins with low cost and the fact that the interior of the egg is guarded by the shell. Also, eggs have high nutritive value, they supply the diet with several essential nutrients, such as zinc, selenium and retinol as well as many minerals and vitamins except vitamin C **(Layman and Rodriguez, 2009).**

The external shell contamination could be important for the shelf life and the food safety of eggs and egg products consumption. It is supposed that bacterial contamination of internal egg content could be the result of penetration of the shell by bacteria deposited on the surface of the egg after it has been laid **(Smith et al., 2000).**

Staphylococcus spp. are most common bacteria contaminating eggshells. Contamination is more likely associated with cracked egg, dirty shells, and contaminated storage. Also, it can be contaminated during formation and laying process **(Abdullah, 2010).**

Staphylococcus spp. are Gram's positive bacteria that can tolerate dry, harsh and salty conditions and present in dust, soil and feces, which is the main reason of its presence on eggshells **(De Reu et al., 2007).** *S. aureus* is a type of bacteria found on human skin, infected cut, eczema, abscesses, wound infections, noses and throats, so it can be transmitted to eggs when handled by a person who has *S. aureus* infection. Also, eggs' contents may be contaminated accidentally by *S. aureus* from shell or it might have originated from ova during egg formation, dust and from the

surface of inanimate objects **(California Egg Commission, 1999).**

Food borne illness is a major public health problem. Staphylococcal food poisoning (SFP) is one of the most common food-borne diseases and results from the ingestion of staphylococcal enterotoxins (Ses) preformed in food by enterotoxigenic strains of *S. aureus*. It is ranked as the third among all foodborne diseases in the world **(Boerema et al., 2006).** Nearly, 50% of *S. aureus* produce enterotoxins which create food intoxication in consumers **(Abdullah, 2010).** Intoxication occurs when the food is contaminated with bacteria producing toxins, like the enterotoxins from *S. aureus*.

There are several types of staphylococcal enterotoxins; A-E, G, H, I and R- T, which are produced either singly or combined by most strains of *S. aureus* **(Argudin et al., 2010).**

The potential health hazards of *S. aureus* is ranged from a variety of self-limiting to life-threatening diseases. The bacteria are a major reason of food poisoning, resulting from the consumption of food contaminated with enterotoxins. Staphylococcal food poisoning (SFP) is characterized by rapid onset of nausea, vomiting, abdominal pain, cramps, and diarrhea. Scalded skin syndrome is caused by exfoliative toxins secreted on the epidermis and mostly affects neonates and young children. Moreover, staphylococcal exfoliative toxins cause other skin conditions such as blisters, pimples, furuncles, impetigo, folliculitis, abscesses and secondary infection **(Fridkin et al., 2005; Eisenstein, 2008).**

Therefore, this study was done to evaluate the potential risk of enterotoxigenic *S. aureus* in table eggs collected from groceries and supermarkets located in El-Fayoum city, Egypt.

2. Materials and Methods

2.1. Collection of Samples

250 table egg samples (75 Baladi hens', 75 white farm hens', 75 brown farm hens' and 25 duck egg samples) were collected randomly from poultry farms, groceries, supermarkets and street vendors in El-Fayoum city, Egypt. Each Baladi hen's egg sample was represented by five eggs, while each farm hen's and duck egg sample was represented by three eggs. Each sample was placed in a sterile plastic bag and transferred to the laboratory without delay whereas they prepared and examined microbiologically.

2.2. Preparation of the Samples

Egg samples were prepared according to **(APHA, 1992).** Eggshell was washed by a surface rinse method and the obtained rinse solution from the five or three eggs of each group was combined. The egg was prepared for evacuation of its content and the contents of each group sample were removed aseptically and received into a sterile mixer until the sample becomes homogenous. Also, the obtained egg content either from five or three eggs of each group was combined to represent one Egg content sample.

2.3. Isolation and Identification of Coagulase Positive *S. aureus* were done According to (ISO/AM 2010)

One ml of both rinsing solution and egg contents samples was transferred to Brain Heart Infusion broth (BHI) and incubated at 35 ºC for 18-24 hours then a loopful from the inoculated BHI broth was spread onto dry surface of Baired Parker agar supplemented with egg yolk tellurite, then the inoculated plate was incubated at 37ºC for 24 to 48 hrs. The suspected colonies were examined for the characteristic *S. aureus* colonial morphology which show circular, smooth, convex, moist, black, shiny with a narrow white margin colonies surrounded by a halo zone extended into the opaque medium. Biochemical tests such as Catalase test, Growth confirmation using Mannitol salt agar and Coagulase test were performed.

2.4. Molecular Identification of Coagulase Positive *S. aureus*

Molecular identification of the isolated strains was done through the detection of coa gene and staphylococcal enterotoxins (Sea, Seb and Sed) genes using PCR and implemented according to **(Iyer and Kumosani, 2011; Mehrotra et al., 2000).**

2.4.1. Extraction of DNA

DNA was extracted using QIA amp DNA Mini Kit. Briefly, 1.5 ml of an overnight broth culture of *S. aureus* grown in Brain Heart Infusion broth (BHI) at 37°C was centrifuged in a benchtop centrifuge at 8000 rpm for 5 min and the supernatant discarded. The cell pellet was re-suspended in PBS to a final volume of 200 ml. QIAGEN protease (20 ml) was pipetted into the bottom of a 1.5 ml micro centrifuge tube then 200 ml of the sample and 200 ml buffer AL were added and mixed by pulse vortexing for 15 seconds. After that, the mixture was incubated at 56˚C for 10 min and centrifuged to remove drops from inside the lid. 200 ml ethanol (96%) were added to the sample and mixed again by pulse vortexing for 15 seconds. After mixing, centrifugation was used to re move drops from inside the lid. The mixture was carefully applied to the QIA amp Mini spin column (in ml collecting tube) for DNA extraction. The DNA concentration was measured using a spectrophotometer. An average of 10 mg of DNA was obtained.

2.4.2. Cycling Conditions of the Primers during PCR

The coa gene and staphylococcal enterotoxins (Sea, Seb and Sed) genes were amplified by a multiplex PCR as described by **(Mehrotra et al., 2000; Iyer and Kumosani, 2011)** as shown in **(Table, 1).** The initial denaturation for Coa gene

was for 5 min at 94°C followed by 35 cycles of 94°C for 30s, 55°C for 40s, 72°C for 45s, and a final extension at 72°C for 10 min. The initial denaturation for staphylococcal enterotoxins (Sea, Seb and Sed) genes were for 5 min at 94°C followed by 35 cycles of 94°C for 30s, 57°C for 40s, 72°C for 45s, and a final extension at 72°C for 10 min. The staphylococcal enterotoxins multiplex PCR Master Mix was prepared according to Emerald Amp GT PCR mastermix (Takara) Code No. RR310A kit.

Running gel electrophoresis of 20 ml of the reaction product in a 1.5% agarose gel (AppliChem, Ottoweg 4 Darmstadt, Germany) at 1–5 volts/cm of the tank length for 30 min and the gel was transferred to UV cabinet and photographed by gel documentation system and the data were analysed using computer software. The DNA was extracted from the positive control reference strains obtained from the National Research Institute, Cairo, Egypt.

3. Results

3.1. Incidence of *Staphylococcus spp.* and Coagulase Positive *S. aureus*

Staphylococcus spp. and coagulase positive *S. aureus* were recovered from eggshells and contents of the examined Baladi, poultry farm (white and brown) and duck eggs samples were illustrated in **Table (2).** Out of 250 examined table egg samples, 52 *Staphylococcus spp.* isolates were recovered with an incidence rate 20.8%. Out of these isolates, 7 strains of *S. aureus* were identified, 3(16.7%) were isolated from Baladi hens' eggs shells and 1(14.3%) was found in white poultry farms' eggs shells, while 2(18.2%) and 1(33.3%) isolates were detected in the examined ducks' egg shells and contents, respectively. PCR results for the coagulase positive *S. aureus* strains (coa gene) was represented in **Fig. (1).**

3.2. PCR for Detection of Staphylococcal Enterotoxin Genes

PCR results for detection of staphylococcal enterotoxins (Sea, Seb and Sed) genes in coagulase- positive *S. aureus* strains recovered from the examined Baladi, poultry farm (white and brown) and duck eggs samples were illustrated in **Table (3)** and **Fig. (2).**

Table (1): Primers sequences used for detection of *coa* and staphylococcal enterotoxins genes.

Gene	Primers sequences	Amplified product	Reference
Coa	ATA GAG ATG CTG GTA CAG G ATA GAG ATG CTG GTA CAG G	Four different types of bands may be detected 350 bp, 430 bp, 570 bp, 630 bp	Iyer and Kumosani (2011)
Sea	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	102 bp	Mehrotra et al., (2000)
Seb	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164 bp	
Sed	CCAATAATAGGAGAAAATAAAAG ATTGGTATTTTTTTTCGTTC	278 bp	

Table 2. Incidence of *Staphylococcus spp.* and coagulase positive *S. aureus* recovered from eggshells and contents of the examined Baladi, poultry farm (white and brown) and duck eggs samples.

Examined samples	No. of examined samples	*Staphylococcus spp.*		Coagulase positive *S. aureus*	
		No.	%	No.	%
Baladi hens' eggshells	75	18	24	3	16.7
White poultry farms' eggshells	75	7	9.3	1	14.3
Brown poultry farms' eggshells	75	4	5.3	0	0.0
Ducks' eggshells	25	11	44	2	18.2
Baladi hens' egg contents	75	6	8.0	0	0.0
White poultry farms' egg contents	75	2	2.7	0	0.0
Brown poultry farms' egg contents	75	1	1.3	0	0.0
Ducks' egg contents	25	3	12.0	1	33.3
Total	250	52	20.8	7	13.5

N.B. Eggshell and egg content are considered one sample.

Table 3. Occurrence of enterotoxins genes (Sea, Seb and Sed) in coagulase- positive *S. aureus* strains recovered from the examined Baladi, poultry farm (white and brown) and duck eggs samples.

Tested samples	*Sea*	*Seb*	*Sed*
One *S. aureus* isolate from Baladi hens' eggshells	-	-	-
Two *S. aureus* isolates from Baladi hens' eggshells	-	+	-
One *S. aureus* isolate from white poultry farms' eggshells	-	-	-
Two *S. aureus* isolates from ducks' eggshells	-	-	-
One *S. aureus* isolate from ducks' egg contents	-	-	+
Total	0	2	1

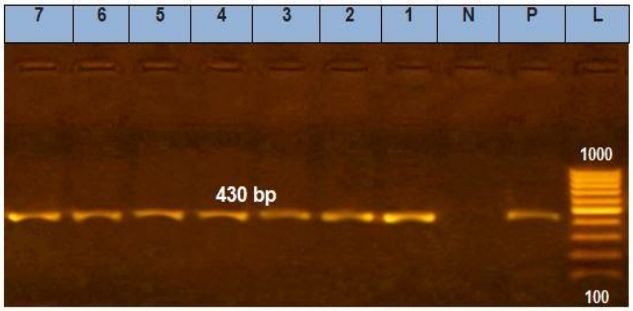

Fig. 1. PCR result for the coagulase positive *S. aureus* strain (coa) gene (430bp). Lane L: DNA ladder, Lane Neg.; control negative, Lane Pos.; control positive, Lane 1, 2, 3, 4, 5, 6 and 7: *S. aureus* isolates.

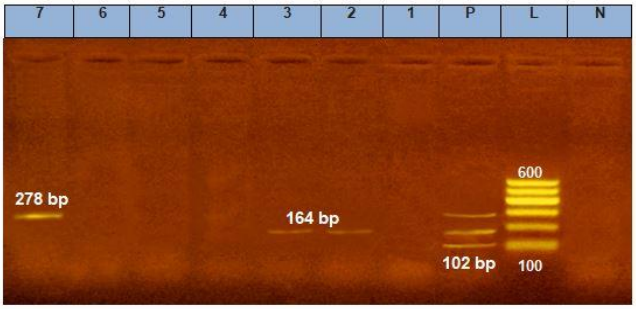

Fig. 2. PCR result for enterotoxin genes Sea (102 bp), Seb (164 bp) and Sed (278 bp) among *S. aureus* Lane L: ladder, lane Pos: control positive, lane Neg: control negative, lane 1-7 (-ve Sea), lane 2 and 3 (+ve Seb), lane 1,4,5,6 and 7 negative Seb) and lane 7 (+ve Sed), lane 1-6 (-ve Sed).

4. Discussion

From data presented in **Table (2),** 18(24%) and 6(8%) *Staphylococcus spp.* isolates were detected in Baladi hens' eggs shells and contents, respectively, while coagulase positive *S. aureus* was isolated from 3(16.7%) and 0(0%) of Baladi hens' eggshells and contents, respectively. Higher incidences of *S. aureus* in shells and contents 48.6% - 17.1% and 40%- 13.3% were obtained by **Refaat (2009)** and **Sadek et al., (2016)** from Baladi hens'' egg shells and contents, respectively. **Awny et al., (2018)** could detect *S. aureus* in 16% of Baladi hens'' eggs contents. However, **Abdel-Tawab (2020)** found *S. aureus* in lower incidence (8%) in Baladi hens'' eggs shells and slightly higher result (2%) from contents.

Summarized results in **Table (2)** proved that *Staphylococcus spp.* were detected in 7(9.3%) and 4(5.3%) from white and brown poultry farms' eggs shells respectively, where it was detected in egg contents in a percentage of 2.7 and 1.3, respectively, while 1(14.3%) of white poultry farms' eggshells were contaminated with *S. aureus.* Although, *S. aureus* wasn't detected in brown poultry farms' shells, white and brown poultry farms' eggs contents. Higher incidences in eggshells (33.3%) and contents (10%) of poultry farms' eggs were reported by **Sadek et al., (2016).** Similar finding of *S. aureus* was reported by **Refaat (2009)** in poultry farms' eggs shells (14.3%). On the other hand, lower result was detected by **Abdel-Tawab (2020)** in poultry farms' eggs shells (4%), but **Awny et al., (2018)** detected *S. aureus* in 52% of poultry farms' eggs contents.

The results obtained in **Table (2)** revealed that Staphylococcus spp. isolates were detected in 11(44%) of the examined Ducks' eggshells samples and 3(12%) of ducks' egg contents. *S. aureus* were detected in 2(18.2%) and 1(33.3%) of the examined ducks' egg shells and contents, respectively. Higher results were obtained by **Refaat (2009)** who recorded that 51.4% and 40% of the examined ducks' eggshells and contents were contaminated with *S. aureus*, respectively, while **Awny et al., (2018)** isolated *S. aureus* from 48% of the examined ducks' egg contents.

The obtained results in our study revealed that the lowest incidence of *S. aureus* was observed in the examined samples of poultry farms hens' eggs which may be attributed to good hygienic measures during production, handling, and storage at farms.

Molecular identification of isolated Coa positive *S. aureus* strains from the examined eggs samples in **Fig. (1),** revealed that all the examined coagulase positive *Staphylococcus spp.* were positive for *S. aureus* Coa gene.

Enterotoxins profiling as illustrated in **Table (3)** and **Fig. (2),** cleared that three 42.86% of Coa positive *S. aureus* strains were showing positive results for the enterotoxins genes. Results revealed that three classical enterotoxin genes (Sea, Seb and Sed) were detected in a percentage of 0.0, 28.6 and 14.3% in Baladi hens' eggshells and ducks' egg contents, respectively. Two isolates of coagulase positive *S. aureus* recovered from Baladi hens' eggshells have Seb gene in percentage of 28.6% but don't have other genes and one isolate was negative for all Ses genes, while one *S. aureus* isolate recovered from white poultry farms' eggshells was negative for all Ses genes. Concerning to ducks' eggs, one coagulase positive *S. aureus* isolate recovered from ducks' egg contents has Sed gene in percentage of 14.3% but 2 isolates recovered from ducks' eggshell were negative for all Ses genes.

Our results agreed with **Fueyo et al., (2001)** who recorded that the detected *S. aureus* isolates by PCR in Spanish eggs were accompanied with production and detection of classical enterotoxin genes. Slightly higher result was detected by **Abdel-Tawab (2020)** who found that Ses were produced by 50% of *S. aureus* isolates, but lower results were obtained by **Kitai et al., (2005)** and **Naffa et al., (2006)** who recorded that Ses were produced by 21.7 and 23% of *S. aureus* isolates, respectively. Moreover, **Yang et al., (2001)** reported the highest levels of Sea (>64 ng/g) and Seb (>64 ng/g) which were produced by *S. aureus* isolates, while **Rasoul et al., (2015)** found Seb in 4.1% of *S. aureus.*

Concerning to the **Egyptian Organization for Standardization and Quality Control (2007)** which mentioned that *S. aureus* must not be found in the egg content. It was noticed that 7 samples failed to achieve the Egyptian Standard levels with incidences of 16.7, 14.3, 18.2 and 33.3 % from Baladi hens' eggshells, white poultry farms' eggshells, ducks' eggshells and ducks' egg contents, respectively as recorded in **Table (2).**

Staphylococcal enterotoxins (Ses) constitute a group of biologically and structurally related toxins. The SEs are the major reason of many outbreaks of food borne diseases and staphylococcal enterotoxin b(Seb) is the most common toxin associated with classic food poisoning **(Lamaita et al., 2005).**

5. Conclusion

This study revealed that the Baladi hens' and ducks' eggs have higher incidences of *Staphylococcus spp.* and coagulase positive *S. aureus* than poultry farm hens' eggs. We recommend that the strict hygienic measures should be adopted in the farms and during production, handling and processing of eggs to protect eggs from being contaminated with pathogenic bacteria causing food poisoning.

6. Authors Contributions

All authors contributed equally to study design methodology, interpretation of results and preparing of the manuscript.

7. Conflict of Interest

The authors declare no conflict of interest.

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